Background: The bone matrix consists of inorganic and organic components and a variety of specialized cells such as osteoblasts, osteocytes and osteoclasts. The bone-forming osteoblasts are responsible for the production of organic matrix components; they differentiate later into osteocytes which is accompanied by mineralization. Osteoclasts are multinuclear giant cells, which resorb bone. Healthy bone homeostasis is characterized by a balanced, dynamic and continuous remodeling process. Glucocorticoids (GCs) are commonly used to successfully treat patients with inflammatory rheumatic and other autoimmune diseases. However, long-term treatment with GC can potentially lead to several adverse effects such as the inhibition of osteoblast proliferation and the increase of osteoclastic activity resulting in osteoporosis.

Objectives: Hence, the aim of our project is to i) develop an in vitro trabecular human bone model, ii) integrate this bone model into a perfusion system to accelerate mineralization and provide biomechanical stimuli and iii) applying prednisolone to induce osteoporosis. Here we present our initial results describing the successful differentiation of osteoblasts and osteoclasts in a 3D environment, and the accomplished integration of the bone model into a perfusion system.

Methods: In a first step, different cultivation conditions were tested to allow optimal osteogenic or osteoclastic differentiation. To this end, a human bone marrow-derived mesenchymal stromal cells (hMSCs) were treated with osteogenic medium, and b) monocytes (isolated from buffy coats) were differentiated into osteoclasts using following protocol: incubation for 3 days with 25 ng/ml RANKL.

Results: The culture of hMSCs was evaluated via Alizarin Red S staining. Osteoclasts were identified using immunofluorescence staining observing multinucleated (DAPI) giant (B-Actin) cells with TRAP and Cathepsin K activity. Additional gene expression analyses are currently conducted using qRT-PCR and looking for osteoclast-specific genes. In parallel to the monolayer cultures, cells were transferred on β-tricalcium phosphate scaffold (βTCP) – a suitable bone-like scaffold. Furthermore, first experiments in a dynamic bioreactor platform (OSPIN GmH) were conducted to evaluate the influence of shear stress on the cells and model systems.

Conclusions: Our results lend support to the potential use of our system for studying osteoporosis. Furthermore, these results might help to develop new therapeutic strategies for the treatment of osteoporosis.

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Objectives: We investigated that BA could suppress RANKL-induced osteoclastogenesis and bone resorption.

Results: BA significantly suppressed osteoclastogenesis by decreasing the phosphorylation of Akt and IκB, as well as PLCγ2-Ca2+ signaling, in pathways involved in early osteoclastogenesis as well as through the subsequent suppression of c-Fos and NFATc1. The inhibition of these pathways by BA was once more confirmed by retrovirus infection of constitutively active (CA)-Akt and CA-IκB retrovirus and measurement of Ca2+ influx. BA also significantly inhibited the expression of osteoclastogenesis-specific marker genes. Moreover, we found that BA administration restored the bone loss induced through acute lipopolysaccharide injection in mice by a micro-CT and histological analysis.

Conclusion: Our findings suggest that BA is a potential therapeutic candidate for bone diseases involving osteoclasts.

Disclosure of Interests: None declared

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FRI0372 INCREASED EXPRESSION OF NOTCH RECEPTORS ON OSTEOCLAST PROGENITORS INDUCED BY RHEUMATOID ARTHRITIS

M. Filipović1, A. Šučur1, D. Flegar1, Z. Jajić2, M. Ikči Matijašević3, N. Lukac1, N. Kovacić1, T. Kelava1, D. Šilić1, K. Zrinski Petrović1, V. Katavić1, D. Grčević1,
1University of Zagreb School of Medicine, Croatian Institute for Brain Research, Laboratory for Molecular Immunology, Zagreb, Croatia, 2University of Zagreb School of Medicine, Clinical Hospital Center “Sestre Milosrdnice”, Department of Rheumatology, Physical Medicine and Rehabilitation, Zagreb, Croatia, 3Clinical Hospital “Sveti Duh”, Department of Clinical Immunology and Allergology, Zagreb, Croatia

Background: Systemic and periarticular bone loss in rheumatoid arthritis (RA) is mediated by osteoclasts, multinucleated cells originating from the myeloid lineage. Recently, Notch signaling pathway has emerged as a potential regulator of osteoclast progenitor (OCP) differentiation and activation.

Objectives: The exact role of Notch signaling in the context of arthritis is still unknown; however, its inhibition has beneficial effects in animal arthritis models.

Methods: Peripheral blood, synovial tissue and subchondral bone marrow were collected from RA patients, and periarticular bone marrow (PBM) and spleen (SPL) were harvested from male C57/Bl6 mice immunized with chicken type II osteoclast progenitor (OCP) differentiation and activation. We aimed to determine the expression of Notch receptors and ligands on specific RA and AS patients. Peripheral blood, synovial tissue and chondrocytes and Notch 1 expressed by chondrocytes and synovial tissue and angiogenic markers.

Results: Peripheral blood, synovial tissue and subchondral bone marrow were collected from RA patients, and periarticular bone marrow (PBM) and spleen (SPL) were harvested from male C57/Bl6 mice immunized with chicken type II collagen. Notch 1 to 4 receptor expression on OCPs was analyzed by flow cytometry. Gene expression of Notch receptors/ligands was determined by qPCR from murine arthritic periarticular tissue by qPCR. During osteoclastogenic culture, murine and human OCPs exhibit a similar gene expression pattern with higher initial expression of Notch 1 and 2, and increase in the expression of Notch 3 and 4 with differentiation. Osteoclasts were also differentiated under Notch-ligand stimulation. Coating with DLL1 results in a greater number of cells expressing osteoclast-specific TRAP, whereas Jag1 seemed to inhibit osteoclastogenesis.

Conclusion: Our results indicate that murine and human OCPs express a distinct tissue-specific pattern of Notch receptors. Notch signaling in OCPs is increased in arthritis and may contribute to the osteoclastogenic potential and increased bone resorption. Our next aim would be to determine the effect of Notch inhibition on OCP activity and arthritis severity.

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FRI0373 ASSOCIATIONS OF VASCULAR PATHOPHYSIOLOGY AND BONE METABOLISM IN ANTI-TNF-TREATED RHEUMATOID ARTHRITIS AND ANKYLOSING SPONDYLITIS PATIENTS

M. Czókolyová1, K. Gulyás1, A. Horváth1, E. Végh1, Z. Petró1, S. Szamosi2, A. Hamar1, A. Pusztai1, E. Balogh1, N. Bodnar1, L. Bodoki1, A. Szentpetery1, H. P. Bhatta1, G. Kerekes1, K. Hodosi1, A. Dömjan1, S. Szántó1, G. Szűcs1, H. Raterman2, W. Lems3, Z. Szekanecz1, 1University of Debrecen, Debrecen, Hungary, 2Northwest Clinics, Alkmaar, Netherlands, 3Amsterdam Rheumatology and Immunology Centre, Amsterdam, Netherlands

Background: Cardiovascular (CV) disease and osteoporosis (OP) have become increasing challenges in the ageing population, even more in patients with inflammatory rheumatic diseases, such as rheumatoid arthritis (RA) and spondyloarthopathies. Both RA and ankylosing spondylitis (AS) have been associated with generalized and localized bone loss, accelerated atherosclerosis, increased CV morbidity and mortality.

Objectives: Bone and vascular biomarkers and parameters along with the effect of one-year anti-TNF therapy on these markers were assessed in order to determine correlations between vascular pathophysiologyme and bone metabolism in RA and AS.

Methods: Fifty-three patients including 36 RA patients treated with etanercept (ETN) or certolizumab pegol (CZP) and 17 AS patients treated with ETN were included in a 12-month follow-up study. Bone and vascular markers were assessed by ELISA. Bone density was assessed by DXA and quantitative CT (QCT). Flow-mediated vasodilation (FMD), common carotid intima-media thickness (cIMT) and pulse-wave velocity (PWV) were assessed by ultrasound. The effects of vascular markers on bone and bone effects on vasculature undergone statistical analysis.

Results: Serum levels of vascular endothelial growth factor (VEGF), PDGF-BB, angiotropin 2 (Ang2) and cathepsin K (CathK) decreased, procollagen type 1 N-propeptide (P1NP) and sclerostin (SOST) levels increased, soluble receptor activator nuclear kappa B ligand (sRANKL) and osteoprotegerin (OPG) levels showed no differences. When bone and vascular markers were correlated with each other, at baseline, OPG correlated with Ang2 and adiponectin. SOST correlated positively with cIMT. DXA L2-4 BMD, DXA L1 BMD and DXA femoral neck (FN) BMD correlated with FMD and CRP. QCT trabecular BMD correlated with cIMT and PON1. According to the univariate analysis, FMD correlated with OPG, colM correlated with SOST and QCT trabecular BMD. Ang1, Ang2 and PDGF-BB showed correlation with Dickkopf-1 (DKK1). Ang2 also correlated with OPG. As suggested by the multivariate analysis, OPG determined FMD; DKK1 was an independent predictor of Ang1, Ang2 and PDGF-BB. OPG was a predictor of Ang2.

Conclusion: In our study of anti-TNF treated RA and AS patients, vascular and bone parameters showed numerous correlations. The therapy was clinically effective, it halted further bone loss over 1 year and reduced the production of angiogenic markers.

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